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PATENT  
Docket No. 432722002621

**CERTIFICATE OF HAND DELIVERY**

I hereby certify that this correspondence is being hand filed with the United States Patent and Trademark Office in Washington, D.C. on November 2, 1999.

*Deborah L. Harley*

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**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

In the application of:

Gregory R. MUNDY et al

Serial No.: 09/421,545

Filing Date: October 20, 1999

For: INHIBITORS OF PROTEASOMAL  
ACTIVITY FOR STIMULATING  
BONE AND HAIR GROWTH



Examiner: Unassigned

Group Art Unit: Unassigned

**COMMUNICATION**

Assistant Commissioner for Patents  
Washington, D.C. 20231

Dear Sir:

As evidenced by the attached copy of the date-stamped postcard, the above-referenced continuation-in-part application was filed with the U.S. Patent and Trademark Office on October 20, 1999, however, page 18 may have been inadvertently omitted when the application was filed.

As noted in the first paragraph of the instant application, "This application is a continuation-in-part of U.S. Serial No. 09/361,775, filed 27 July 1999 which is a continuation-in-part of U.S. Serial No. 09/113,947 filed 10 July 1998. The contents of both of these applications are incorporated herein by reference." Therefore, if page 18 is indeed missing from the

Application, Applicants respectfully request that the Patent Office insert the attached page 18 into the application as if it were filed with the Application on October 20, 1999.

Applicants do not believe that any fees are due at this time; however, if the Patent Office determines that an extension and/or other relief is required, applicants petition for any required relief including extensions of time and authorizes the Assistant Commissioner to charge the cost of such petitions and/or other fees due in connection with the filing of this document to Deposit Account No. 03-1952.

Respectfully submitted,

Dated: November 2, 1999

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diseases. While topical administration is generally preferable for stimulating hair growth, as generally only local effects are desired, systemic treatment may be preferable in some instances as well.

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#### Assays for Compounds Useful in the Invention

Assays for assessing the ability of a compound to inhibit proteasomal activity and for inhibitors of NF- $\kappa$ B activity are well known in the art. Two typical, but nonlimiting assays are described below.

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#### Assessment of Proteasomal Activity

Proteasomal inhibition activity is most conveniently measured by the assay described in Example 5 hereinbelow. The assay involves incubating the potential inhibitor with 20S thermophila proteasomes which, in purified form, are commercially available, with a fluorogenic peptide substrate. The presence of an inhibitor will reduce 15 the amount of fluorescence generated by the action of the proteasome fraction on the fluorogenic peptide. This assay is described in further detail in Coux, O. *et al.* *N Rev Biochem* (1996) 65:801; Adams, J. *et al.* *Cancer Res* (1999) 59:2615; and Craiu, A. *et al.* *J Biol Chem* (1997) 272:13437. Further reports are set forth in Hilt, W. *et al.* *Trans Biochem Sci* (1996) 21:96; Peters, J. *Trends Biochem Sci* (1994) 19:377; Maupin-Furlow, 20 J.A. *et al.* *J Biol Chem* (1995) 270:28617; and Jensen, T.J. *et al.* *Cell* (1995) 83:129. Fluorogenic substrates and purified proteasomes are available, for example, from CalBiochem, San Diego, CA.

#### NF- $\kappa$ B Activity Assays

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Cells are treated with different concentrations of compounds, and nuclear extracts prepared. Briefly, cells are washed with phosphate-buffered saline, and resuspended in lysis buffer (0.6% Nonidet P-40, 150 mM NaCl, 10 mM Tris-HCl, pH 7.9, 1 mM EDTA, 0.5 mM DTT and a cocktail of protease inhibitors (Complete (TM), Boehringer Mannheim). After incubation on ice for 15 min, nuclei are collected by centrifugation.



ATTORNEY DOCKET 432722002621 DATE October 20, 1999

APPLICANT Gregory R. Mundy et al.

TITLE: INHIBITORS OF PROTEASOMAL ACTIVITY FOR STIMULATING BONE  
AND HAIR GROWTH

PAPERS ENCLOSED: BOX PATENT APPLICATION

<input checked="" type="checkbox"/> SPECIFICATION	<u>43</u> PAGES	<input checked="" type="checkbox"/> DECLARATION (unexecuted)
<input checked="" type="checkbox"/> CLAIMS	<u>3</u> PAGES	<input type="checkbox"/> ASSIGNMENT WITH RECORDATION
<input checked="" type="checkbox"/> ABSTRACT	<u>1</u> PAGES	<input checked="" type="checkbox"/> CERTIFICATE OF <del>EXPRESSION</del> <sup>HAND CARRY</sup> MAIL
<input checked="" type="checkbox"/> DRAWINGS	<u>2</u> SHEETS	<input type="checkbox"/> VERIFIED STATEMENT
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